2. LITERATURE REVIEW

2.1 Microorganisms in meat

2.1.1 Meat spoilage and pathogenic microorganisms

Many factors affect the storage life of fresh meat and the keeping quality of meat and poultry products. It can be predicted by monitoring for spoilage microorganisms (Gill and Bryant, 1992). Also temperature plays a vital role in meat spoilage (Narashimha Rao *et al*., 1998) and is considered most important. Based on temperature requirements, microorganisms are classified as psychotrophs, mesophiles and thermophiles (Table 2.1).

Table 2.1: Cardinal temperatures for microorganisms (Narashimha Rao, 1998)

Group	Temperature (ºC)		
	Minimum	Optimum	Maximum
Thermophiles	40 – 45	55 – 65	60 – 90
Mesophiles	5 – 10	30 – 45	35 – 47
Psychrotrophs	-5 – +5	25 – 30	30 – 45
Psychrophiles	-5 – +5	12 – 15	15 – 20

Meat is recognized as a source of several bacterial pathogens that cause food poisoning in humans although the source of infection is not determined in the majority of outbreaks of food-borne infectious disease investigated (Hinton, 2000). There are several reasons for this, an important one being that the food responsible for the problem has usually been consumed completely, or has been disposed of before microbiological investigations are instituted.

There are three important factors determining the microbiological quality of the meat sold by the butchers: the condition of the animal slaughter, the spread of

contamination during slaughter and processing, and the temperature, time and other conditions of storage and distribution (Nortje *et al.*, 1990).

Currently the most important pathogens associated with raw meat are *Campylobacter* spp., *Clostridium perfringens*, and pathogenic serotypes of *Escherichia coli,* for example *E. coli* O157:H7, *Salmonella* and certain serotypes of *Yersinia enterocolitica*. *Listeria monocytogenes* is also a common contaminant of meat. The most important microorganisms associated with the meat of different animals are shown in Table 2.2 (Borch *et al.*, 1996; Qiongzhen *et al.*, 2004; Nel *et al.*, 2004). Many of these bacteria are confined to the intestinal tract of the animal, while others occur, for example, in the nasopharynx or on the skin. All of them may contaminate carcasses during dressing and further handling (Yashoda *et al*., 2000).

Usually, the organisms are capable of prolonged survival on meat surfaces, although *C. jejuni* is sensitive to drying. With the exception of spores of clostridia and aerobic bacilli, food-borne pathogenic bacteria are heat sensitive and should be killed by proper cooking, especially when present as surface contaminants.

In the process of pig slaughter a wide range of potential pathogens, such as *Salmonella* (Currier *et al.*, 1986; Borch *et al.*, 1996; Berends *et al.*, 1996), and *Listeria monocytogenes* (Reij and Aantrekker, 2004; Borch *et al*., 1996; Nel, *et al*., 2004) can contaminate the surface of carcasses. There are many opportunities for carcass contamination to occur during slaughter. The main emphasis of control is applied at the end of evisceration in the form of washing. Nevertheless, the initial scalding and singeing steps that are performed to de-hair carcasses have also been demonstrated to remove a substantial proportion of the carcass surface microflora (Borch *et al.*, 1996; Warriner, *et al.*, 2002); and can be considered to act as barriers to minimize the transfer of pathogens through the line. However, for more effective control of pathogen spread there is a need to develop a hazard analysis critical control point scheme within the pig slaughter process (Gill and John, 1997; Goodfellow, 1995).

Many reports have been published that highlight the potential for carcass contamination during dehairing and evisceration operations (Gill and Bryant 1993; Pearce *et al.*, 2004; Swanenburg *et al.*, 2001a). Such studies have been based on enumerating total aerobic and indicator organism counts from samples recovered from carcasses (Nel *et al.*, 2004; Palumbo *et al.*, 1999). However, although such methods permit the gross changes in carcass microflora to be determined, this does not provide sufficient data to elucidate the origins of pathogens. In addition, as pathogens typically occur in low numbers, contamination of carcasses is not necessarily reflected by an increase in bacterial counts.

Table 2.2: Pathogens of primary concern in raw meat and poultry (Pearson and Dutson, (1995).

Meat	Pathogen
Poultry:	*Salmonella*
	Campylobacter jejuni
	L. monocytogenes
	C. perfringens
	C. botulinum
Pork:	*Salmonella*
	Yersinia enterocololitica
	Campylobacter jejuni
	L. monocytogenes
	C. perfringens
	C. botulinum Trichenella spiralis
	Toxoplasma gondii
Beef:	*Salmonella*
	Escherichia coli O157:H7
	L. monocytogenes
	C. perfringens
	C. botulinum

2.1.2 *Enterobacteriaceae*

Members of the genera belonging to the *Enterobacteriaceae* family have been placed among the most pathogenic and most often encountered organisms in clinical and food microbiology (Quinn *et al.*, 1998; Miliotis and Bier, 2003). These gram-negative straight rods are usually associated with intestinal infections, but can be found in almost all natural habitats. They are the causative agents of such diseases as dysentery, typhoid, and food poisoning.

All members of this family are oxidase negative, glucose fermenters and nitrate reducers. In most cases, the pathogenicity of a particular enteric bacterium can be determined by its ability to metabolize lactose. Non-utilizers are usually pathogenic while the lactose utilizers are not (Holt *et al*., 2000).

They are distributed worldwide, they found in soil, water, fruits, vegetables, grains, flowering plants and trees, and animals from worms and insects to humans (Holt *et al*., 2000). There is substantial heterogeneity in the ecology, host range, and pathogenic potential to humans and animals, insects, and plants. A number of species cause diarrheic diseases including typhoid fever and bacillary dysentery. Many species not normally associated with diarrheic diseases are often referred to as opportunistic pathogens (Holt *et al*., 2000). Most of these, as well as the species causing diarrheic disease, can cause a variety of extra-intestinal infections including meningitis, bacteremia in the urinary and respiratory tracts, as well as wound infection.

Enterobacteriaceae are often used as hygiene indicators of foods of animal origin (Anon., 2001; Crowley *et al.*, 2005; Warriner *et al.*, 2002; Nel *et al.*, 2004; Zweifel *et al.*, 2005). Their presence on processed food may give a better indication than coliforms of inadequate treatment or post-process contamination from the environment, and may help to indicate the extent of fecal contamination (Anon., 2001). However, the greatest application of *Enterobacteriaceae* and other indicator

organisms is the assessment of the overall quality of a food and the hygiene conditions present during the food processing.

Various sampling methods have been utilized to determine the number of bacteria on the surface of food processing equipment and red meat animal carcasses (Palumbo *et al.*, 1999). The principal sampling methods are swabbing and excision; in addition rinse techniques, contact (Rodac) plates and different tape methods have been used (Pearce, *et al.*, 2005). Each has its advantages and disadvantages. Because they are easier to use, require the least amount of specialized material and provide data, which are generally more reproducible, swabbing and excision have found the widest acceptance and use.

A possible procedure for objectively assessing the hygienic performance of the carcass dressing process with respect to both safety and storage stability has been proposed. The procedure involves the collection of swab samples from randomly selected sites on randomly selected carcasses at appropriate points at the end of a process (Gill *et al.*, 1996).

At the abattoir, *Enterobacteriaceae* and pseudomonads were the biggest contributors to psychotrophic count, at the wholesalers' the *Enterobateriaceae* and micrococci counts, and at the retailers' the micrococci and pseudomonads respectively (Nortje *et al.*, 1990). This indicates the *Enterobateriaceae* might be common psychotrophs in the meat production chain, originating from the abattoir and from the environments.

The presence of *Enterobacteriaceae* in meat or meat products indicates possible fecal contamination (Pearce, *et al.*, 2005). The steps in pig slaughter that lead to an increased *Enterobacteriaceae* count are dehairing, polishing, and evisceration. Scalding and singeing are steps, which result in considerable decrease in the numbers of microorganisms on carcass surfaces. After singeing, the surface is probably almost free of *Enterobacteriaceae,* and evisceration leads to the recontamination of carcasses

with *Enterobacteriaceae* (Morgan *et al*., 1987 and Berends *et al*., 1996), since after singeing there are no steps that lead to a decrease in numbers of bacteria.

Aerobic and *Enterobacteriaceae* counts are used as indicator organisms in meat and food products. A high APC on carcasses usually indicates the degree of care taken during slaughter and unsuitable time or temperature conditions during the production and storage of the meat. It can also indicate heavy post-slaughter and post-processing contamination. The counts of *Enterobacteriaceae* and *E. coli* have been used as an indicator of direct contamination of carcasses with fecal material. The detection of such microorganisms on carcasses could also indicate indirect contamination from the intestinal tract during slaughter (McEvoy *et al.*, 2004; Nel *et al.*, 2004; Zweifel *et al.*, 2005; Byrne *et al.*, 2005). In the European Union (Anon. 2001) it is recommended that the routine analysis should be based on Total Viable Count and *Enterobacteriaceae* (Table 2.3)

Table 2.3: Daily log mean value for bacterial performance criteria for cattle, sheep, goats, horses and pigs according to the Commission Decision 2001/471/EU.

	Acceptable Range (m)		Marginal Range (>m but ≤ M	Unacceptable Range (M)
	cattle/sheep/ goats/horses	pigs	cattle/pigs/sheep/ goats/horses	cattle/pigs/sheep/ goats/horses
Total viable counts (TVC)	< 3.5 log	< 4.0 log	3.5 log (pig: 4,0 log) - 5.0 log	>5.0 log
Enterobacteriaceae	< 1.5 log	< 2.0 log	1.5 log (pig: 2,0 log) – 2.5 log (pig:3.0 log)	> 2.5 log (pig: > 3.0 log)

2.1.3 *Salmonella* spp.

Taxonomy

The genus *Salmonella*, family *Enterobacteriaceae,* is comprised of anaerobic, facultative anaerobic, catalase-positive gram-negative rod-shaped bacteria and contains two species: *Salmonella enterica* and *Salmonella bongori*, based on the phenotypic criteria (D' Aoust *et al.*, 2001). As shown in Table 2.4, the species *S. enterica* is divided into six subspecies: subspecies *enterica* (I), subspecies *salamae* (II), subspecies *arizonae* (IIIa), subspecies *diarizonae* (IIIb), subspecies *houtenae* (IV), and subspecies *indica* (VI). The actual number of serovars in all *Salmonella* species and subspecies is 2501 (Popoff, 2004). Most isolates of *Salmonella* from warm-blooded animals belong to the subspecies *enterica* (I). The other subspecies are found in cold-blooded animals and in the environment.

The nomenclature for the genus *Salmonella* has evolved from the initial one serotype-one species concept proposed by Kaufmann (D' Aoust *et al.*, 2001). Serotype identification, delivered from agglutination reactions with specific antisera, is based upon the organism's component of somatic (O antigen), capsular, and flagella (H antigen) antigens. The **O antigens** are the lipopolysaccharides (LPS) of the outer membrane, similar to the O antigens of other *Enterobacteriaceae*. The **H antigens** are the proteins that make up the peritrichous flagella of the bacteria; they can be expressed in one of two forms (termed phases) (D' Aoust *et al.*, 2001).

Phase1 H antigen is specific and associated with the immunological identity of that serovar. However, *Salmonella* strains can alter flagella antigens to phase2 (containing a different antigenic subunit protein), which is shared by many serovars. Certain *Salmonella* express a surface-bound polysccharide capsular antigen, which typically blankets the O antigen and blocks O-agglutination; however, the capsular can be selectively removed by heat treatment prior to O-agglutination assay. The virulence (Vi) capsular antigen occurs in *Salmonella* serovars Typhi, Paratyphi C and Dublin (D' Aoust *et al*, 2001) upon primary isolation.

Table 2.4: *Salmonella* species, subspecies, numbers of serotypes in each subspecies, and their usual habitats (Popoff *et al.*, 2004).

Salmonella species and subspecies	No. of serotypes	Usual habitat Within subspecies
S. enterica subsp. *enterica* (I)	1,478	Warm-blooded animals
S. enterica subsp. *salamae* (II)	498	Cold-blooded animals and the environment
S. enterica subsp. *arizonae* (IIIa)	94	Cold-blooded animals and the environment
S. enterica subsp. *diarizonae* (IIIb	327	Cold-blooded animals and the environment
S. enterica subsp. *houtenae* (IV)	71	Cold-blooded animals and the environment
S. enterica subsp. *indica* (VI)	12	Cold-blooded animals and the environment
S. bongori (V)	21	Cold-blooded animals And the environment
Total	2,501	

Serotype names designated by antigenic formulae include the following: (i) subspecies designation (subspecies I through VI), (ii) O (somatic) antigens followed by a colon, (iii) H (flagellar) antigens (phase 1) followed by a colon, and (iv) H antigens (phase 2, if present) (for example, *Salmonella* serotype IV 45:g,z51:2. For formulae of serotypes in S. bongori, V is still used for uniformity (for example, S. V 61:z35:2).

The name usually refers to the geographic location where the serotype was first isolated. For named serotypes, to emphasize that they are not separate species, the serotype name is not italicized and the first letter is capitalized (Table 2.5). At the first citation of a serotype the genus name is given followed by the word “serotype” or

the abbreviation “ser.” and then the serotype name (for example, *Salmonella* serotype or ser. Typhimurium). Subsequently, the name may be written with the genus followed directly by the serotype name (for example, *Salmonella* Typhimurium or *S.* Typhimurium (Popoff *et al.*, 2000; Popoff and Le Minor, 1997). Both versions of the serotype name are listed as key words in manuscripts to facilitate the search and retrieval of information on *Salmonella* serotypes from electronic databases.

Table 2.5: *Salmonella* nomenclature in use in literatures.

Taxonomia position	Nomenclature
Genus (italics)	*Salmonella*
Species (italics)	• *enterica*, which includes subspecies I, II, IIIIa, IIIb, IV and V • *bongori* (formerly subspecies V)
Serotype (capitalized, not italicized)	• The first time a serotype is mentioned in the text; the name should be preceded by the word “*serotype*” or “ser.” • Serotypes are named in subspecies I and designed by antigenic formulae in subspecies II to IV, and VI and S. bongori • Member of subspecies II, IV and VI and *S. bongori* retain their names if named before 1966

Table 2.6: Examples of antigenic structure formulae for some common Salmonellae, modified from Krieg and Holt (1984).

Serovars	Somatic	Antigens		Combination
	(O) Antigens	Phase 1	Phase 2	
	Group 02 (A)			
S. Paratyphi *A*	1,2,12	A	[1,5]	1,2,12:a:1,5
S. Nitra	2,12	g,m	-	2,12:g,m:-
	Group 04 (B)			
S. Kisangani	1,4,[5],12	A	1,2	1,4,5,12:a:1,2
S. Canada	4,12	B	1,6	4,6:b:1,6
S. Derby	1,4,12	f,g	[1,2]	1,4,12:f,g:1,2
S. Agona	1,4,[5],12	f,g,s	-	1,4,5,12:g,f,s:-
	Group 06,7 (C_1)			
S. Paratyphi C	6,7[Vi]	C	1,5	6,7:c:1,5
S. III arizonae	6,7	-	1,6	6,7:-:1,6
	Group 09,12(D_1)			
S. Endai	1,9,12	A	1,5	1,9,12:a:1,5
S. Typhi	9,12[Vi]	D	-	9,12,Vi:d:-
S. Enteritidis	1,9,12	g.m	[1,7]	1,9,12:g,m:1,7
	Group 03,10 (E_1)			
S. Aminatu	3,10	A	1,2	3,10:a:1,2
S. Amsterdam	3,10	g,m,s	-	3,10:g,m,s:-
	Group O67			
S. Crossness	67	R	1,2	67:r: 12

Symbols: [ ], may be absent; ( ) not well developed (weakly agglutinable). The underlined antigens are associated with phage conversion.

Salmonellosis in pigs

Salmonellosis is an important cause of human gastroenteritis in western countries (Danilo *et al*., 2000). Pork contaminated with *Salmonella* is recognized as one of the causes of human salmonellosis. Pigs are an important reservoir of *Salmonella* for humans. Infection of man follows either through direct contact or more frequently concludes from pork and pork products (Feddorka-Cray *et al.*, 2000). Pigs can become infected with *Salmonella* at the breeding and/or fattening farm (van der Wolf *et al.*, 1999 and 2001). However, from the moment the pigs leave the farm, there are also many opportunities to become infected or contaminated with *Salmonella* during transport, lairage or slaughter (Warriner *et al.*, 2002; Botteldoorn *et al.*, 2003; Søren *et al.*, 2003). This implies that control measures taken on the farm in order to decrease *Salmonella* prevalence should be combined with measures to prevent pigs and pork from contamination after the pigs have left the farm.

Infected pigs remain healthy carriers in most of the cases and as a consequence are of great importance to public health. *Salmonella* infections in swine are of concern for two reasons. The first is the clinical disease in pigs (salmonellosis), and the second is that pigs are susceptible to infection with a broad range of *Salmonella* serotypes constituting a potential source of human exposures and illness (Schwartz, 1998).

Recent investigations have shown that *Salmonella* could be isolated from 23% of finishing pig herds in the southern part of the Netherlands (Van der Wolf *et al.*, 1999), and from 26% of rectal samples of slaughtered pigs (Swanenburg *et al.*, 2001a,b). In Europe and the USA, the predominant not species-adapted *Salmonella* serovars found in pigs are *S.* Typhimurium and *S.* Derby. In Germany, the most common serovar following *S.* Typhimurium in 1961–1965 was *S.* Dublin and in 1998 it was *S*. Agona, while in Denmark it was *S.* Infantis (Feddorka-Cray *et al.*, 2000). In England in 1997, of the 338 *Salmonella* incidents reported in pigs, 62% were caused by *S.* Typhimurium, and 12% by *S.* Derby. In Denmark, 6.2% of fecal samples were found positive, usually with one phage type predominating from each farm source

(Baggsen *et al.*, 1996). From the isolated species-adapted *Salmonella* serovars *S.* Choleraesuis was the most common strain recovered.

Salmonella in pigs at slaughter

Pigs can become infected with *Salmonella* during transport and lairage due to stress, mingling with salmonellae excreting pigs, and contact with a *Salmonella*-contaminated environment, if the truck/lairage was not cleaned and disinfected well (Berends *et al.*, 1996; Morgan *et al.*, 1987; Isaacson *et al.*, 1999; Swanenburg *et. al*., 2001a). It has been shown that the proportion of pigs in a herd that excrete *Salmonella* increased after transport (Isaacson. *et al.*, 1999). Fedorka-Cray *et al.* (1995) showed that *Salmonella* could be isolated from mesenterial lymph nodes and caecal and rectal contents already 3 hours after infection, which makes it possible for pigs to pick up *Salmonella* during transport or in lairage, and start excreting before they are slaughtered. In this way, they can infect other pigs, as well as the environment of the truck and lairage. Carcasses can become contaminated with *Salmonella* during the slaughter procedure by contaminated slaughter equipment (Oosterom *et al.*, 1985; Gill and Bryant, 1993; Sammarco *et al.*, 1997). A study by Käsbohrer *et al*. (2000) from seven abattoirs located in different states of Germany reported that *Salmonella* were isolated from 3.7% of the fecal samples, 3.3% of the lymph nodes and 4.7% of the surface swabs.

Salmonella prevalence in slaughter pigs has been investigated in many parts of the world. *Salmonella* could be isolated from portal lymph nodes, mesenterial lymph nodes or rectal contents. Swanenburg *et al.*, (2001a,b) reported that *Salmonella* could be isolated from either rectal contents (26.5%), tonsils 19.6%, and 9.3% in mesenterial lymph nodes, livers and tongues of slaughtered pigs. Oosterom *et al.* (1985) found *Salmonella* in the intestinal tract of 21% of slaughtered pigs and on 13% of carcasses after evisceration.

Results from other countries showed prevalences of *Salmonella* in samples of slaughtered pigs that sometimes differed from each other. Finlay *et al.* (1986) isolated

Salmonella from 2% of muscle samples and 3.7% of fecal samples of slaughtered pigs in Canada. Currier *et al.* (1986) isolated *Salmonella* from 13.5% of fecal samples in the USA, whereas Letellier *et al.* (1999) isolated *Salmonella* from 5.2% of fecal samples in Canada. Morse and Hird (1984), Lammerding *et al.* (1988), Keteran *et al.* (1982) and Lazaro *et al.* (1997) isolated *Salmonella* from 4.3% USA, 14.2% Canada, 31.3% USA and 40% (Brazil) of mesenteric lymph nodes, respectively. Lazaro *et al.* (1997) isolated *Salmonella* from 77.5% of tonsils of slaughtered pigs. Unfortunately, these results are hard to compare, because *Salmonella* isolation procedures and kinds of samples collected differed among these different studies.

The number of *Salmonella* organisms on the surfaces of carcasses of pigs may be reduced as a result of careful slaughter procedures, such as scalding individually, careful removal of intestines (Oosterom and Notermans, 1983; Berends *et al.*, 1997), a plastic bag over the rectum (Nesbakken *et al.*, 1994; Sørensen *et al.*, 1999), and a decontamination step after slaughter (Snijders et al, 1985; Snijders, 1988). Separate slaughter of pigs free from a certain pathogen, to avoid introduction of certain bacterial zoonoses into the slaughter-line and to avoid cross-contamination between herds during slaughter (Swanenburg *et al.*, 2001a,b). Maffia *et al.* (1989) and Sammarco *et al.* (1997) both investigated the slaughterhouse environment and concluded that *Salmonella* can be present on floors and working tables. Oosterom and Notermans (1983) showed that fewer pigs were contaminated with *Salmonella* after slaughter in The Netherlands if they were singed individually and the guts were removed carefully.

2.2 Possible sources of microorganisms in meat

Muscles of healthy animals are free from microorganisms because of defensive mechanisms associated with skin and mucous membranes, hair and cilia, gastric juice, the intestine and urine. Inflammatory processes and humoral antibodies play a role (Narasimha Rao *et al*. 1998). All these defence mechanisms present barriers to the entry of microorganisms into the muscles of live animals. Microorganisms inevitably gain access to meat at slaughter when the defences break down, and also during processing. So, minimization of microbial contamination is essential in meat handling systems in order to retard meat spoilage as well as to prevent health hazards that may arise from meat consumption. Therefore there is a need to know how microorganisms enter meat and to determine critical control points of contamination.

Microorganisms contaminating meat are derived from the environment (soil and water), gastrointestinal contents, hide, skin, or feathers of animals, processing equipment and personnel. A survey performed by the WHO (1995) in Europe indicated that 25% of the food-borne outbreaks could be traced back to recontamination. The most important factors contributing to the presence of pathogens in processing food were insufficient hygiene (1.6%), cross-contamination (3.6%), processing and storage in inadequate rooms (4.25%), contaminated equipment (5.7%), and contamination by personnel (9.2%).

Sources of microbial contamination in fresh meat have been documented (Gill and Lander, 2004; Lo Fo Wong *et al.*, 2002; Nel *et al.*, 2004; Gill *et al.*, 1998; Mossel *et al.*, 1998). Hides and skin, hooves, fleece and hair of live animals, gut microflora, the sticking-knife, scalding tank, equipment, instruments and tools (overhead rail, gambrels, stainless steel platforms, s-hooks, trays, tables, knives, axes, saw blades), chopping blocks (wooden), floor, walls, air, water, cloths, hands and boots have been identified as sources of microbial contamination of carcasses and meat cuts (Warriner *et al.*, 2002; Botteldoorn *et al.*, 2003). Bacteria can contaminate meat during the following operations: sticking, skinning, scalding, de-hairing, evisceration, and splitting and quartering.

During post-mortem meat inspection, palpation and incision of lymph nodes, infected tissues or tissues with abnormalities can give rise to cross contamination. Incision should be avoided where possible, and palpation of organs should be as minimal as possible (Borch *et al.*, 1996). Pathogenic bacteria that will subsequently be transferred to the carcass are likely on contaminated knives, cutters and other tools/equipments used.